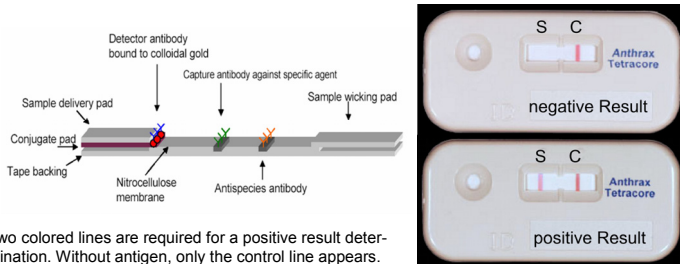


Since the anthrax letters in 2001, hand-held assays (HHAs) have been controversially discussed as a quick screening method for first responders. The HHAs from the American company Tetracore for the detection of anthrax, plague and tularemia were evaluated and tested for sensitivity (limit of detection), specificity (selectivity, cross-reactivity) and repeatability.

Introduction

The test strip is coated with lyophilised antibodies which are reconstituted by adding the sample solution. One of the specific antibodies is labeled with a colloidal gold derivative and it binds to specific antigen. This complex moves along the strip membrane by capillary action. In the S window the complex is captured and immobilised by a second specific antibody. In this area the gold-label accumulates and forms a visible colored line. In the C window, free gold-labeled antibodies are captured and a second band appears indicating that the test strip functioned properly.



Two colored lines are required for a positive result determination. Without antigen, only the control line appears.

Method

All strains were cultivated on appropriate media. The concentrations of vegetative and spore suspensions were determined by viable plate counts. The stock solutions were serially diluted immediately before use. The assays were performed according to the manufacturer's instructions for liquid samples. All tests were performed in triplicates. The results were read visually after 15 minutes.

Biosafety

Anthrax, plague and tularemia strains were handled in the BSL-3 Glovebox system at SPIEZ LABORATORY.

Results

Sensitivity

The sensitivity of an assay is the minimum number of microorganisms required to give a positive test result. The limit of detection is the concentration at which three samples out of three were positive.

Limit of detection:

Strain	Limit of detection
<i>B. anthracis</i> A 58	10 ⁷ spores/ml
<i>B. anthracis</i> A 1 ("Stierne")	> 10 ⁷ spores/ml
<i>B. anthracis</i> A 73202.2000 ("Pasteur")	10 ⁷ spores/ml
<i>B. anthracis</i> W 514	10 ⁷ spores/ml
<i>Y. pestis</i> CO 92 (37°C)	3 x 10 ⁴ cells/ml
<i>Y. pestis</i> NCTC 02868 (37°C)	3 x 10 ⁵ cells/ml
<i>Y. pestis</i> NCTC 10030 (37°C)	3 x 10 ⁶ cells/ml
<i>F. tularensis</i> ATCC 6223	5 x 10 ⁷ cells/ml
<i>F. tularensis</i> Schu 4 (inactivated)	10 ⁷ cells/ml

Specificity

Specificity refers to the ability of an assay to accurately discriminate between a target microorganism and other bacterial species or other matrices. To determine specificity, each assay was tested for cross-reactivity with a variety of different bacterial species and interfering substances.

Specificity of the anthrax assay:

Sample	Concentration	Test Result Positive/Total
Five different <i>B. anthracis</i> strains (vegetative)	10 ⁶ cells/ml	3/3
<i>B. anthracis</i> NCTC 05444 (vegetative)	10 ⁶ cells/ml	0/3
<i>Bacillus thuringiensis</i>	10 ⁷ spores/ml	0/3
<i>Bacillus subtilis</i>	10 ⁷ spores/ml	0/3
<i>Bacillus cereus</i>	10 ⁶ cells/ml	0/3
<i>Escherichia coli</i>	7 x 10 ⁶ cells/ml	0/3
<i>Enterococcus faecalis</i>	3 x 10 ⁶ cells/ml	0/3
<i>Yersinia enterocolitica</i>	6 x 10 ⁶ cells/ml	0/3
<i>Staphylococcus aureus</i>	9 x 10 ⁶ cells/ml	0/3
salt	1 g/ml	3/3
sugar	1 g/ml	invalid
flour	1 g/ml	0/3
soil	1 g/ml	0/3
<i>B. anthracis</i> A 58 / salt	2 x 10 ⁷ spores/g	3/3
<i>B. anthracis</i> A 58 / sugar	2 x 10 ⁷ spores/g	invalid
<i>B. anthracis</i> A 58 / flour	2 x 10 ⁷ spores/g	3/3
<i>B. anthracis</i> A 58 / soil	2 x 10 ⁷ spores/g	1/3

B. anthracis strain NCTC 05444 ("St. Mary's") was false negative at 10⁶ cells/ml. There was no cross-reactivity with other species, however, the spore concentrations of the *Bacillus* species were determined after the fact to be below the limit of detection for *B. anthracis*. Salt produced false positive results and there were matrix effects with sugar and soil.

Specificity of the plague assay:

Sample	Concentration	Test Result Positive/Total
Six different <i>Y. pestis</i> strains (28°C)	3 x 10 ⁷ cells/ml	0/3
<i>Yersinia enterocolitica</i>	10 ⁷ cells/ml	0/3
<i>Yersinia pseudotuberculosis</i>	3 x 10 ⁷ cells/ml	0/3
<i>Yersinia ruckeri</i>	10 ⁷ cells/ml	0/3
<i>Yersinia kristensenii</i>	10 ⁷ cells/ml	0/3
<i>Yersinia intermedia</i>	10 ⁷ cells/ml	0/3
<i>Yersinia mollaretii</i>	10 ⁷ cells/ml	0/3
<i>Yersinia bercovieri</i>	10 ⁷ cells/ml	0/3
<i>Yersinia aldovae</i>	10 ⁷ cells/ml	0/3
<i>Yersinia frederiksenii</i>	10 ⁷ cells/ml	0/3
<i>Bacillus cereus</i>	3 x 10 ⁷ cells/ml	0/3
<i>Escherichia coli</i>	3 x 10 ⁷ cells/ml	0/3
<i>Enterococcus faecalis</i>	10 ⁷ cells/ml	0/3
<i>Staphylococcus aureus</i>	2 x 10 ⁷ cells/ml	0/3
<i>Bacillus subtilis</i>	6 x 10 ⁷ spores/ml	0/3
<i>Bacillus anthracis</i> A58	10 ⁷ spores/ml	0/3
salt or sugar or flour or soil	1 g/ml	0/3
<i>Y. pestis</i> CO92 (37°C) / salt or sugar or flour or soil	2 x 10 ⁶ cells/g	3/3

All six strains grown at 28 °C generated false-negative results. The target epitope in this assay is presumably the antigen F1 which is only expressed at 37 °C. No cross-reactivity, matrix effects or interferences were observed.

Specificity of the tularemia assay:

Sample	Concentration	Test Result Positive/Total
<i>Bacillus anthracis</i> (inactivated)	10 ⁷ spores/ml	0/3
<i>Yersinia pestis</i> (inactivated)	10 ⁷ cells/ml	0/3
<i>Bacillus cereus</i>	3 x 10 ⁶ cells/ml	0/3
<i>Escherichia coli</i>	3 x 10 ⁶ cells/ml	0/3
<i>Enterococcus faecalis</i>	10 ⁷ cells/ml	0/3
<i>Staphylococcus aureus</i>	2 x 10 ⁷ cells/ml	0/3
salt or sugar or flour or soil	1 g/ml	0/3
<i>F. tularensis</i> Schu 4 / salt or sugar or flour or soil	10 ⁷ cells/g	3/3

No cross-reactivity, matrix effects or interferences were observed.

Repeatability

The repeatability of an assay reflects its ability to produce the same results under the same test conditions. With the anthrax assay 64 out of 66 triplicates were identical. With the plague assay 51 out of 51 triplicates and with the tularemia assay 21 out of 21 triplicates gave identical results.

Conclusion

For all three assays, the limit of detection depends on the individual strain and is much higher than the quantity reported to produce diseases.

- The use of the anthrax assay cannot be recommended because hoax substances can easily generate false-positive responses.
- Also the plague assay cannot be recommended because it is incapable of detecting highly infectious *Y. pestis* when grown at 28°C. Due to slightly better growth this is the proposed incubation temperature in the laboratory. The F1 antigen is an unsuitable target for detection.
- The tularemia assay is sufficient as a quick screening method with the limitation that it does not exclude a health hazard.

Clue question: Is bad information worse than no information?



BSL-3 Glovebox system at SPIEZ LABORATORY

